

Chemically Competent *E. coli* Transformation:

Materials Needed:

Competent DH5α *E. coli* cells
Plasmid
LB
Water bath
LB with antibiotic plates
Eppendorf tubes
Pipets

Protocol:

1. Take competent DH5α *E. coli* cells from -80°C. In an Eppendorf tube, put 50µL for transforming a DNA construct or 100 µL for transforming a ligation. More or less cells are required depending on how competent the cells are. Keep the tubes on ice
2. Turn on water bath to 42°C
3. Add 1 µL of circular DNA into the *E. coli* cells.
4. Incubate on ice for 10 minutes
5. Place tube(s) with DNA and *E. coli* into the 42°C water bath for 45 seconds
6. Place tubes back on ice for 2 minutes to reduce damage to the *E. coli* cells
7. Add 1 mL of LB (with no antibiotic added) into each tube.
8. Incubate tubes for 30 minutes to 1 hour at 37°C, chose the latter if transforming a ligation
9. Spread about 100 µL of the resulting culture on LB plates (with appropriate antibiotic added) Grow overnight and pick colonies once grown.

References

1. Teruel, M. (2003). Transformation Protocol Using Heat Shock. Stanford databases.
Retrieved from <http://web.stanford.edu/~teruel1/Protocols/>.